PB90-135559

Anaerobic Dechlorination of 2,4-Dichlorophenol in Freshwater Sediments in the Presence of Sulfate

(U.S.) Georgia Univ., Athens

Prepared for:

Environmental Research Lab., Athens, GA

Oct 89

U.S. Department of Commerce
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1. REPORT NO.	2.	3. PB90-135559
EPA/600/J-89/255		

4. TITLE AND SUBTITLE
ANAEROBIC DECHLORINATION OF 2,4-DICHLOROPHENOL IN FRESHWATER SEDIMENTS IN THE PRESENCE OF SULFATE

5. REPORT DATE

6. PERFORMING ORGANIZATION CODE

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8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
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10. PROGRAM ELEMENT NO.
CBWD1A

11. CONTRACT/GRANT NO.
CR812638

12. SPONSORING AGENCY NAME AND ADDRESS
Environmental Research Laboratory - Athens, GA
Office of Research and Development
U.S. Environmental Protection Agency
Athens GA 30613

13. TYPE OF REPORT AND PERIOD COVERED
Journal article

14. SPONSORING AGENCY CODE
EPA/600/01

15. SUPPLEMENTARY NOTES

Applied and Environmental Microbiology 55(10):2735-2737, October 1989

16. ABSTRACT

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2 → con't to ps 2735

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	4 5
	20. SECURITY CLASS *(This page)*	22. PRICE
	UNCLASSIFIED	

EPA/600/J-89/255

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 1989, p. 2735–2737
0099-2240/89/102735-03$02.00/0
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Vol. 55, No. 10

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Anaerobic Dechlorination of 2,4-Dichlorophenol in Freshwater Sediments in the Presence of Sulfate

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Received 17 March 1989/Accepted 18 July 1989

In the presence of added sulfate, 2,4-dichlorophenol and 4-chlorophenol were transformed stoichiometrically to 4-chlorophenol and phenol, respectively, in anaerobic freshwater lake sediments between 18 and 40°C. The concomitantly occurring sulfate reduction reduced the initial sulfate concentration from 25 mM to about 6 to 8 mM and depressed methane formation.

Some toxic compounds, especially polychlorinated aromatic compounds, are slowly degraded in polluted aerobic zones and, therefore, may leach into anaerobic subsurface environments. If not degraded there, these compounds will pollute aquifers. Sulfate and nitrate function as electron acceptors in the anaerobic environments; the addition of these anions thus has the potential to alter the carbon flow and the interactions among members of the microbial community. Both anions may be present in the soil or may be introduced concomitantly with the organic pollutants. It is presently believed that shifting to sulfate-reducing conditions, e.g., by adding sulfate to a methane-producing freshwater sediment, will prevent reductive dehalogenation. The anaerobic biodegradation of aromatic compounds in sulfate-reducing environments has been studied mainly in anaerobic marine sediments and sewage sludge (1–10, 13, 15). Gibson and Suflita (7) compared samples from a freshwater aquifer which contained methanogenic and sulfate-reducing sites. They found that only their methanogenic site exhibited dechlorination. Although samples from the sulfate-reducing site exhibited degradation of phenol and benzoate, no chlorophenols or chlorobenzoates were transformed within 3 months of incubation. We have studied the anaerobic biodegradation of 2,4-dichlorophenol over a wide temperature range under methanogenic conditions in freshwater lake sediments from Georgia (12). These sediments also contained mesophilic and thermophilic sulfate reducers (unpublished results). We report here that in our samples from freshwater sediments which had been amended with sulfate, 2,4-dichlorophenol and 4-chlorophenol were reductively dehalogenated while sulfate reduction occurred.

The anaerobic sediments were mainly obtained from a pond at Sandy Creek Nature Center near Athens, Ga. (for a description of sediment and sampling methods, see reference 12). The sediment exhibited a redox potential of less than −300 mV before and after the samples were obtained and processed. The sulfate concentrations of unamended samples varied between 0.5 and 15 ppm (0.005 and 0.15 mM). Sodium sulfate (25 mM) or sodium nitrate (25 mM) was added to the sediments from anaerobic stock solutions and then thoroughly mixed. Sulfate concentrations in the amended sediments were determined by ion chromatography (Dionex 2010i with an AS-4A Dionex column and a mobile phase consisting of 2.8 mM $NaHCO_3$–2.2 mM Na_2CO_3). The data reported here on transformations and methane formation are based on repeated experiments carried out in parallel samples with two independently collected samples from two different locations.

In the sulfate-amended samples, 130 μmol of 2,4-dichlorophenol per liter (27 ppm) disappeared completely when the samples were incubated at between 19 and 40°C. Examples of required degradation times are 50, 90, and 366 days at 22, 28 (Fig. 1), and 40°C, respectively. No dechlorination was observed below 18 and above 40°C within 366 days (Fig. 2B). However, methanogenesis, measured as an indicator of anaerobic microbial activity, occurred above 40°C (Fig. 2C). The lack of dechlorination in the presence of the added sulfate above 40°C contrasts with the results obtained with samples not amended with sulfate (12).

In the absence and presence of added sulfate, 2,4-dichlorophenol was stoichiometrically transformed to 4-chlorophenol after a temperature-dependent adaptation period. In the presence of added sulfate, the adaptation periods for 2,4-dichlorophenol transformation to 4-chlorophenol (Fig. 2A)

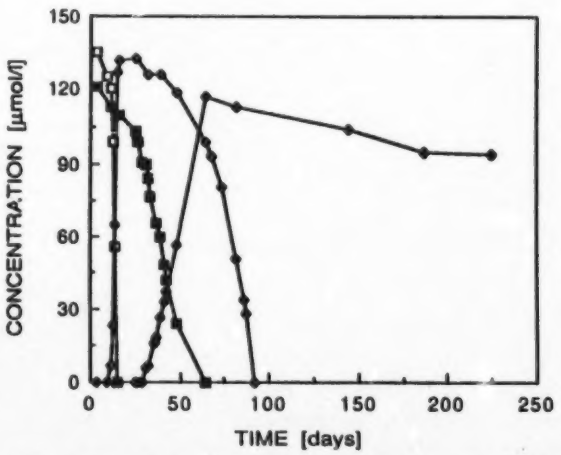

FIG. 1. Degradation of 2,4-dichlorophenol at 28°C under methanogenic and sulfate-reducing conditions. Symbols: □, 2,4-dichlorophenol; ◇, 4-chlorophenol; ■, 2,4-dichlorophenol plus sulfate; ◆, 4-chlorophenol plus sulfate.

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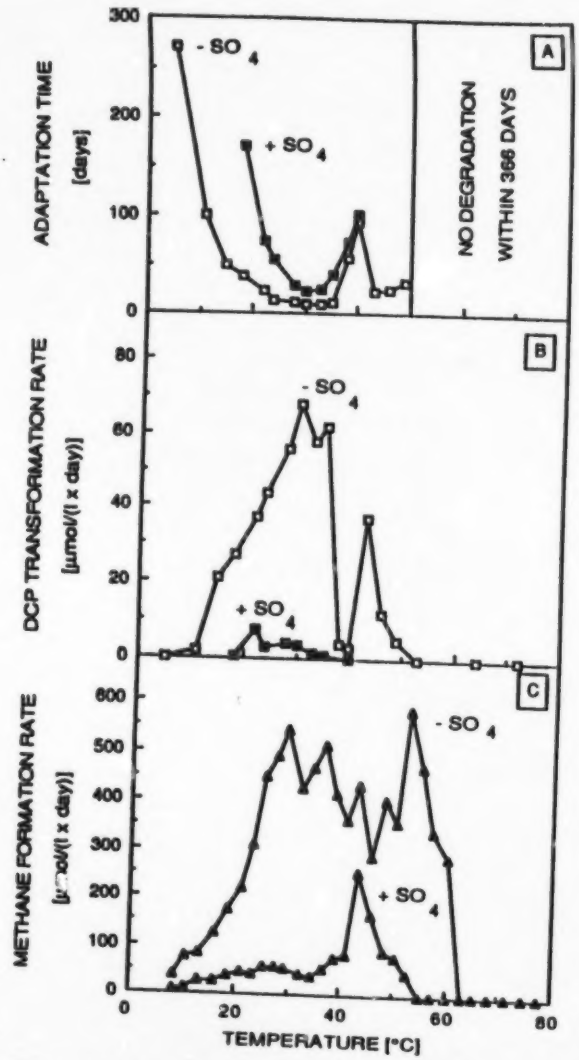

FIG. 2. Temperature effect on 2,4-dichlorophenol dechlorination adaptation time (A), 2,4-dichlorophenol (DCP) transformation rate (B), and methane formation rate (C) under methanogenic and sulfate-reducing conditions. The reported transformation rate values were corrected for the nonbiological disappearance of the parent compound in autoclaved (40 min at 121°C, repeated on three consecutive days) controls at the respective temperatures (12). Data shown are similar to data obtained from repeated experiments carried out in parallel samples (the differences between the two parallels were within the size of the symbols) with two independently collected sediment samples.

were longer than those found under methanogenic conditions (12), and the dechlorination rates were notably lower (Fig. 2B) at corresponding temperatures. No further transformation of the product 4-chlorophenol was observed within 1 year (a representative example for 28°C is shown in Fig. 1) in these experiments. The slight disappearance observed is assumed to be an artifact caused by adsorption into the butyl rubber stoppers, as observed with sterile controls (12).

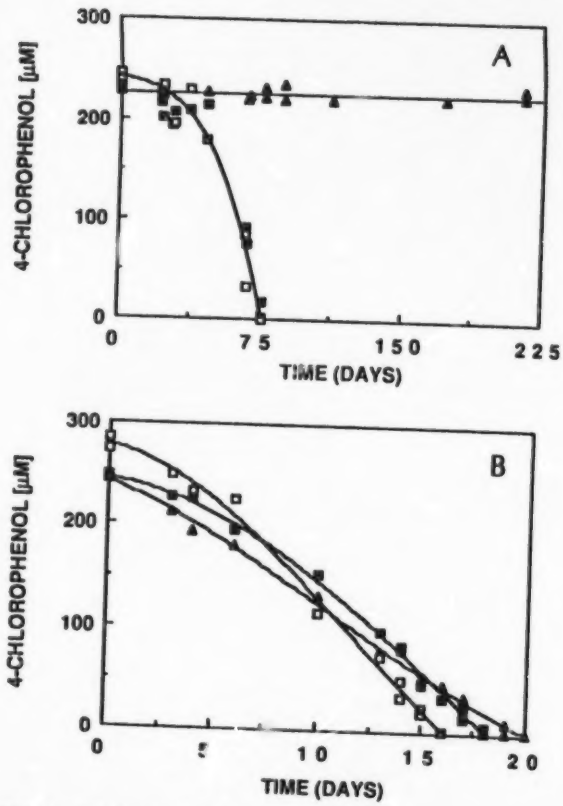

FIG. 3. Dehalogenation of 4-chlorophenol under sulfate-reducing conditions. (A) Unadapted sediment samples; (B) Sediments adapted for dechlorination under methanogenic conditions. Sodium sulfate concentrations (wt/vol) used were 0% (□), 0.25% (■), and 0.5% (▲).

However, when 4-chlorophenol together with sodium sulfate (at various concentrations; Fig. 3) was added to nonadapted sediments or to sediment adapted to 4-chlorophenol dechlorination under methanogenic conditions, transformation occurred at 30 to 31°C (the only temperatures tested). In the sediments collected later, the addition of 0.5% (wt/vol; about 50 mM) sulfate slowed down the dehalogenation by about 25% (Fig. 3B) and a 4-chlorophenol dechlorination rate of up to 2 ppm (16 µmol/liter) per day was obtained under the sulfate-reducing conditions. Unlike the controls (i.e., samples to which no sulfate was added) in the 0.25 and 0.5% sulfate-amended samples, the total amount of methane formed while dechlorination occurred decreased to less than 0.5% of the control. Concomitantly, the sulfate concentration was reduced, e.g., from 0.25% (wt/vol; 25 mM) sulfate to between 0.06 and 0.08% (wt/vol; 6 and 8 mM). These concentrations were considered sufficient to ensure that sulfate-reducing conditions were maintained during the entire experiment, as indicated by the nearly total inhibition of methane formation. Methane formation for all amended and nonamended samples was determined as a control measurement. At most temperatures, methanogenesis was inhibited by the addition of sulfate, as expected (11, 14, 16). However, at temperatures around 44°C, the rate of methanogenesis was not greatly affected (Fig. 2C). Interestingly, in addition to methane formation, sulfate reduction also occurred at this

temperature (data not shown). Presently, we have no experimentally proven explanation for the absence of the usually observed competition.

Because no organisms which can reductively dechlorinate 2,4-dichlorophenol or 4-chlorophenol have been isolated in pure cultures, it is not known whether the dechlorinations under methanogenic and sulfate-reducing conditions are catalyzed by the same organisms. However, the increased transformation rate for 4-chlorophenol under sulfate-reducing conditions after adaptation under methanogenic conditions indicates that the same organism could be catalyzing the dechlorinations under both conditions. This assumption agrees with the finding (D. Dalton, personal communication) that high transformation rates of 2,4-dichlorophenol to 4-chlorophenol were obtained in stable enrichment cultures containing no methanogens. Unlike the aquifer samples studied by Gibson and Suflita (7), the dechlorinating organisms in our sample might compete successfully with the sulfate reducers for hydrogen.

This is the first report of dechlorination of 4-chlorophenol and 2,4-dichlorophenol in freshwater sediments in the presence of sulfate reduction. However, in naturally 2,4-dibromophenol-adapted marine sediments, King (10) observed dechlorination of 2,4-dichlorophenol. In our anaerobic samples from a sea grass bed near the mouth of a mangrove channel at Crab Cay, Bahamas, we did not find dechlorination at temperatures ranging between 5 and 72°C. This indicates that not all marine sediments, especially those with no or few 2,4-dibromophenol-producing organisms, are adapted to degrade chloro-substituted aromatic compounds.

The addition of 0.15% (wt/vol; about 25 mM initial concentration) nitrate to the dechlorinating or freshly collected freshwater sediment samples inhibited the dechlorination of 2,4-dichlorophenol during an incubation period of 130 days at temperatures between 5 and 72°C. Nitrate was reduced and N_2 was produced. No methane formation was detectable in these samples. This contrasts with the finding of Genthner et al. (B. R. Sharak Genthner, W. A. Price, and P. H. Pritchard, Abstr. Annu. Meet. Soc. Environ. Toxicol. Chem. 1987, abstr. no. 415), who detected transformation of monochlorophenols in nitrate-amended samples.

We thank J. W. Fitzgerald and M. E. Watwood for the sulfate analysis and J. E. Rogers for helpful discussions.

We thank R. E. Hodson as well as Capt. Morgan and the crew of the ORV *Columbus Iselin* for giving us the opportunity to do the experiments at Chub Cay Island (National Science Foundation grant OCE-84-16384 to R. E. Hodson). This work was supported by U.S. Environmental Protection Agency grant CR 812638 and in part by Department of Energy grant DE-FG 09-86 13614 to J. Wiegel.

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